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(54) Title: A NOVEL THERAPY FOR TREATING SEPSIS USING A SOLUBLE FORM OF RECOMBINANT CD14 MYELOMONOCYTIC ANTIGEN

(57) Abstract

A method is provided for the treatment of symptoms of sepsis that are mediated by proteins that are substantially homologous to CD14 myelomonocytic antigen. A soluble form of CD14 ("sCD14") is produced by using molecular genetic techniques to express a nucleic acid sequence that encodes CD14. The soluble form of the CD14 thus produced is isolated and purified. The purified soluble CD14 is administered to mammals in order to prevent or treat the symptoms of sepsis.

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A NOVEL THERAPY FOR TREATING SEPSIS USING A SOLUBLE FORM OF RECOMBINANT CD14 MYELOMONOCYTIC ANTIGEN

FIELD OF THE INVENTION

This invention relates generally to the development of methods and therapies for effectively preventing or treating the symptoms of medical conditions such as sepsis and tissue rejection, and for studying the cellular and molecular mechanisms that result in sepsis.

BACKGROUND OF THE INVENTION

Sepsis is a life-threatening medical condition that can be brought on by infection or trauma. The symptoms of sepsis can include chills, profuse sweating, fever, weakness, or hypotension, followed by leukopenia, intravascular coagulation, shock, adult respiratory distress syndrome, multiple organ failure, and often, death. R. Ulevitch, et al., J. Trauma 30: S189-92 (1990).

The symptoms of sepsis can be induced by certain substances (elements, molecules, chemical compounds, or any mixture thereof) liberated during infection or trauma. Some pathogenic bacteria, viruses, and plants elaborate sepsis-inducing substances.

The lipopolysaccharides ("LPS"; also, "endotoxins") that are typically present on the outer membrane of all gram-negative bacteria are among the most studied and best understood sepsis-inducing substances. While the precise chemical structures of LPS molecules obtained from different bacteria may vary in a species-specific fashion, a region called the lipid A region is common to all LPS molecules. E. Rietschel et al., in Handbook of Endotoxins, 1: 187-214, eds. R. Proctor and E. Rietschel, Elsevier, Amsterdam (1984). This lipid A region mediates many, if not all, of the LPS-dependent pathophysiologic changes that characterize sepsis.

LPS is believed to be a primary cause of death in humans afflicted with gram-negative sepsis. van Deventer et

al., Lancet, 1: 605 (1988); Ziegler *et al.*, J. Infect. Dis., 136: 19-28 (1987). Treatment of patients suffering from sepsis and gram-negative bacteremia with a monoclonal antibody against LPS decreased their mortality rate. Ziegler *et al.*, N. Eng. J. Med., 324: 429 (1991). LPS and gram-negative bacteria may also play a role in the pathology of auto-immune conditions such as Reiter's syndrome, which is associated with rheumatoid arthritis.

LPS causes polymorphonuclear leukocytes, endothelial cells, and cells of the monocyte/macrophage lineage to rapidly elaborate and release a variety of cell products, among these immunoregulatory substances known as cytokines that are capable of initiating, modulating or mediating humoral and cellular immune responses and processes.

One particular cytokine, alpha-cachectin or tumor necrosis factor (TNF), is apparently a primary mediator of septic shock. Beutler *et al.*, N. Eng. J. Med., 316: 379 (1987). Intravenous injection of LPS into experimental animals and man produces a rapid, transient release of TNF. Beutler *et al.*, J. Immunol., 135: 3972 (1985); Mathison *et al.*, J. Clin. Invest. 81: 1925 (1988). Pretreatment of animals with anti-TNF antibodies reduces lethality, suggesting that TNF is a critical mediator of septic shock. Beutler *et al.*, Science, 229: 869, (1985); Mathison *et al.*, J. Clin. Invest. 81: 1925 (1988).

Molecular receptors that can combine with sepsis inducing substances, and that once combined, initiate certain chemical reactions play a critical role in the etiology of the symptoms of sepsis. Several monocyte/macrophage surface antigens that possess receptor and signal transduction functions have been identified. Many of them are cell differentiation markers (i.e., they are characteristically present only in defined stages, especially the end stages, of cells of a defined lineage and function).

One such marker, CD14, is a 55-kD glycoprotein expressed strongly on the surface of monocytes and

macrophages, and weakly on the surface of granulocytes such as neutrophils. 5. M. Goyert *et al.*, J. Immunol. 137: 3909 (1986); A. Haziot *et al.*, J. Immunol. 141: 547-552 (1988); S. M. Goyert *et al.*, Science 239: 497 (1988). CD14 is linked by a cleavable phosphoinositol tail [A. Haziot *et al.*, J. Immunol. 141: 547-552 (1988)] to the exoplasmic surface of mature monocytes, macrophages, granulocytes and dendritic reticulum cells, of renal nonglomerular endothelium, and of hepatocytes in rejected livers. A soluble form of CD14 is present in normal sera and in the urine of nephrotic patients. Bazil *et al.*, Eur. J. Immunol. 16: 1583 (1986).

The characteristic cell type- and stage-specific expression of CD14 in mature cells of the myelomonocytic lineage suggests an important effector function. Antibodies to CD14 reduced human monocyte chemiluminescence, caused the internalization of CD14 molecules, and caused transient increases in interleukin-1 synthesis, cytosolic calcium concentration and monocyte H_2O_2 production. F. Lund-Johansen *et al.*, FEBS Lett. 273: 55 (1990). Interleukin 4 has been shown to down-regulate the expression of CD14. R. Lauener, S. Goyert, R. Geha and D. Vercelli, Eur. J. Immunol. 20: 2375 (1990). Together, these observations suggest that CD14 may possess an intrinsic, regulatable capacity to engage in signal transduction.

The observation that certain conditions such as hyperthermia [M. Kappel *et al.*, Clin. Exp. Immunol. 84: 175 (1991)] or tissue rejection [J. Bogman *et al.* Lancet, 238: *ii* (1989)] lead to the proliferation of CD14-positive monocytes, suggests that CD14-bearing cells are important elements in the immune response to these medical conditions. Immunostaining with anti CD14 monoclonal antibodies is capable of differentiating rejection from other forms of interstitial nephritis, and has been used to diagnose renal allograft rejection. J. Bogman *et al.* Lancet, 238: *ii* (1989). In a recently published preliminary report, CD14 was detected on the surface of hepatocytes in 6 out of 8 cases of liver allograft rejection, but not in ten cases of acute and

chronic hepatitis due to virus infection, autoimmunity, or drugs. R. Volpes *et al.*, Lancet, 337: 60 (1991). In addition to demonstrating for the first time the epithelial expression of CD14, this last result correlates the hepatocellular expression of this antigen with liver allograft rejection.

In vitro analyses have shown that CD14 is the receptor for lipopolysaccharide (LPS or endotoxin) when LPS is bound to an acute phase serum protein called LBP (LPS binding protein). LBP recognizes the lipid A region of LPS and forms high affinity 1:1 stoichiometric complexes. Tobias *et al.*, J. Biol. Chem., 264:10867 (1989). The binding of this complex to CD14 causes cells to become highly activated and release interleukins, tumor necrosis factor ("TNF"), H_2O_2, and other substances which eventually cause the lethal symptoms observed during sepsis, including the "shut-down" of the cardiovascular-pulmonary-renal systems. Beutler *et al.* N. Eng. J. Med., 316:379 (1987); R. Ulevitch *et al.*, J. Trauma 30:189-92 (1990); F. Lund-Johansen et *al.*, FEBS Lett. 273: 55 (1990).

The cDNAs and the genes for human and murine CD14 have been cloned and sequenced. E. Ferrero and S. M. Goyert, Nuc. Acids Res. 16: 4173 (1988); S. M. Goyert *et al.*, Science 239: 497 (1988); M. Setoguchi *et al.*, N. Nasu, S. Yoshida, Y. Higuchi, S. Akizuki, and S. Yamamoto, Biochem. Biophys. Acta 1008: 213-22 (1989). The sequence analysis revealed that CD14 belongs to a family of leucine-rich membrane-bound and soluble proteins that have receptor and cell adhesive functions. M. Setoguchi *et al.*, Biochem. Biophys. Acta 1008: 213-22 (1989); E. Ferrero, C.L. Hsieh, U. Francke and S.M. Goyert, J. Immunol. 145: 133 (1990).

The human CD14 protein sequence contains five potential sites for N-linked glycosylation and contains a 10 fold repeat of a leucine rich motiff (LXXLXLX). There is a 66% amino acid sequence identity between the murine and human CD14s. The murine gene is located on mouse chromosome 18, which like the human gene also contains at least five genes encoding receptors. M. Setoguchhi, N. Nasu, S. Yoshida, Y.

Higuchi, S. Akizuki, and S. Yamamoto, Biochem. Biophys. Acta 1008: 213-22 (1989); E. Ferrero, C.L. Hsieh, U. Francke and S.M. Goyert, J. Immunol. 145: 133 (1990).

In situ chromosomal hybridization of the ^{3}H-labelled cDNA probe to normal human metaphase cells resulted in specific labeling only of chromosome 5. S. M. Goyert *et al.*, Science 239: 497 (1988). The labeled sites were clustered at regions 5q22-q32 of this chromosome. The largest cluster of grains was located at 5q23-q31. S. M. Goyert *et al.*, Science 239: 497 (1988). This region of human chromosome 5 is known to contain a cluster of genes that encode several myeloid-specific growth factors or growth factor receptors, as well as other growth factor and receptor genes. S. M. Goyert *et al.*, Science 239: 497 (1988). The mapping of the CD14 gene to this region of chromosome 5, its expression preferentially by mature myeloid cells, and its deletion in the malignant cells of patients having myeloid leukemias and del(5q) suggest that the CD14 antigen may play a role in the pathogenesis of myeloid disorders.

For the preceding reasons, it is an object of this invention to develop methods and therapies for the effective treatment, including prevention, for symptoms of sepsis in those individuals afflicted by symptoms of sepsis. It is also an object of this invention to develop methods and therapies for the effective protection of individuals who are at risk of becoming afflicted by the symptoms of sepsis.

It is a further object of the invention to provide methods and means for studying the mechanisms of ailments such as sepsis, as a model for diseases caused by host immune response to exogenous and endogenous triggers of the immune system. Such methods and means expressly include methods for the testing of substances that cause, mediate or prevent symptoms of sepsis. ("Cause" includes beginning molecular events that result in the symptoms of sepsis or are implicated in the organism's response to sepsis; "mediate" includes any molecular events that form part of the causal

chain of events that result in the symptoms of sepsis or are part of the organism's response to sepsis.)

Finally, it is an object of this invention to develop methods and therapies for the effective treatment, including prevention, of symptoms of tissue rejection and of auto-immune disease that are mediated by LPS, gram-negative bacteraemia, and/or CD14.

SUMMARY OF THE INVENTION

A new therapy has been developed for treatment of the symptoms of sepsis that are mediated by the CD14 receptor. A soluble form of the human CD14 ("sCD14") receptor was produced by using molecular genetic techniques to express an isolated nucleic acid sequence that encodes human CD14 in a Baculovirus expression system. The soluble form of the human CD14 was isolated and purified. When injected into mice exposed to endotoxin (also, "LPS"), the sCD14 protected the against the lethal effects of endotoxin.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 demonstrates that either a monoclonal antibody to human CD14 (MoS34) or soluble recombinant CD14 itself can inhibit the LPS-induced activation of monocytes or neutrophils; figure 2 is a picture of an SDS-polyacrylamide gel of the purified sCD14 isolated from culture supernantants of Sf9 cells.

DETAILED DESCRIPTION OF THE INVENTION

A. Isolation and characterization of the human CD14 gene.

A human cDNA library was constructed in pCD, the OkayamaBerg eukaryotic expression vector [H. Okayama and P. Berg, Mol. Cell Biol. 3: 280 (1983)] using messenger RNA (mRNA) isolated from human M4-AML (myelomonocytic) cells. S. M. Goyert *et al.*, Science 239: 497 (1988). 1.0 to 2.65 kb cDNA inserts were size-selected in low-melting agarose gels according to T. Yokota *et al.*, Proc. Natl Acad. Sci. 81: 1070 (1984). S. M. Goyert *et al.*, Science 233: 497 (1988). *Escherichia coli* (RR1) were then transformed with the cDNA and plated on agar. A total of 1056 colonies were randomly selected, transferred individually to small liquid cultures,

and grown overnight at 37°C. The 1056 liquid cultures were consolidated into 44 pools of 24 liquid cultures each. Each pool was grown in 500 ml of Luria broth containing 100 μg of ampicillin per milliliter, and plasmid DNA was isolated from each pool and used to transfect COS 7 cells as described by S. M. Goyert *et al.*, Science 239: 497 (1988).

The COS 7 cells transfected with the 44 plasmid pools were analyzed for cell surface expression of CD14 by indirect immunofluorescence using a monoclonal antibody (MoS39) to CD14, [Dimitiu-Bona *et al.*, J. Immunol. 130: 145 (1983)] and a fluoresceinated sheep antibody to mouse immunoglobulin. Five of the clones derived from these pools were positive for human CD14 expression. S. M. Goyert *et al.*, Science 239: 497 (1988).

Each of the 24 plasmids from one of the positive pools was isolated on a cesium chloride gradient, transfected individually into COS 7 cells, and screened for CD14 expression as described above. One cDNA clone, labelled pCD-CD14, was found to express CD14. S. M. Goyert *et al.*, Science 239: 497 (1988).

To confirm that the pCD-CD14 clone encoded authentic CD14 molecules, immunoprecipitates prepared from pCD-CD14-transfected COS 7 cells and from M4-AML cells expressing endogenous CD14 were compared by SDS-polyacrylamide gel electrophoresis. The molecules precipitated from both sources were nearly identical in size. S. M. Goyert *et al.*, J. Immunol. 137: 3909 (1986). In addition, the pCD-CD14 probe was found to hybridize to a single mRNA species that showed an expression profile identical to CD14: it was present in monocytes, granulocytes and M4-AML cells, but not in less mature myeloid cells represented by the leukemic cell lines k62 (undifferentiated), U937 (monoblast-like), HL60 (promyelocytelike), or M2-AML (myeloblastic with maturation) cells or lymphocytes. S. M. Goyert et al., Science 239: 497 (1988). The predicted protein sequence of the pCD-CD14

clone corresponded to the partial protein sequence of CD14 determined by microsequence analysis.

The pCD-CD14 cDNA clone was found to consist of 1367 nucleotides with a polyadenylate tail at the 3' end. S. M. Goyert *et al.*, Science 239: 497 (1988). An initiation codon was identified at position 105, followed by an open reading frame (coding region) consisting of 1125 nucleotides flanked by 104 nucleotides of 5' untranslated sequence and 126 nucleotides of 3' untranslated sequence. Comparison with the partial protein sequence determined by microsequence analysis confirms the identity of this clone as encoding CD14 and indicates the presence of a signal peptide of 19 amino acids (-19 to -1).

The human CD14 gene was isolated from a size-selected (6 kb average) Eco RI genomic library constructed in the lambda vector gtWes. S. M. Goyert et al., Science 239: 497 (1988). DNA sequence analysis demonstrated that the human CD14 gene contains a single intron of 88 base pairs immediately after the ATG translational start site. E. Ferrero and S. M. Goyert, Nuc. Acids Res. 16: 4173 (1988). The initiation codon is flanked by a sequence which shows homology to the consensus sequence $C(C)^{A}_{G}CCATCC$ for a translation initiation site [as defined by M. Kozak, Nucl. Acids Res. 15: 8125-8148 (1987)] and is separated from the rest of the coding region by the 88 bp intron.

Southern blot analysis of DNA digested with several different restriction enzymes and probed with CD14 cDNA gave single bands, suggesting that CD14 is encoded by a single gene. S. M. Goyert *et al.*, Science 239: 497 (1988).

B. Production and purification of soluble recombinant CD14.

Recombinant CD14 was produced using the Baculovirus expression system. G.E. Smith *et al.*, Proc. Natl. Acad. Sci. USA, 82: 8404 (1985); V. Luckon and M. Sommers, Biotechnology 6: 47 (1988). Briefly, an NCO1 and MSE1-cut restriction fragment that contained the human CD14 cDNA was cloned into the BluePac Transfer vector and co-transfected into Sf9

insect cells along with the wild-type AcNPV virus. The resulting recombinant virus, produced by homologous recombination between the recombinant plasmid and the wildtype virus, yielded approximately 8 mg of sCD14 per liter of serum-free media.

The recombinant protein was purified by affinity chromatography and analyzed by SDS-PAGE. Recombinant CD14 is seen to be comprised of multiple bands (probably due to glycosylation intermediates) and is smaller in size (45 kD) than native soluble CD14 (55 kD). The identity of the multiple bands was confirmed by Western blot. (Figure 2.)

For the functional studies described below, the affinity-purified material was subsequently treated with protein A to remove any contaminating monoclonal antibody which might have eluted from the affinity column. Contaminating LPS was removed with polymyxin B beads. Following dialysis against endotoxin-free PBS, the recombinant CD14 was filtered, aliquoted and stored at (-)20°C. The amount of endotoxin remaining after dialysis was found to be negligible (less than 10 ng/ml).

C. Soluble CD14 inhibits the LPS-induced activation of monocytes and neutrophils *in vitro* in a dose-dependent fashion. (Figure 1)

The effect of sCD14 and of a monoclonal antibody to human CD14 on the LPS-induced activation of immune system cells was assessed by incubating monocytes or neutrophils in the presence of different concentrations of complexes ("LPS:LBP") composed of lipopolysaccharide ("LPS") and serum LPS-binding protein ("LBP"), in the absence or presence of either sCD14 or of the monoclonal antibody.

The LPS was obtained from commercial sources (Sigma). LBP was purified from rabbit acute phase serum according to Tobias *et al.* J. Exp. Med. 164:777 (1986). LPS:LBP complexes were formed in sterile polypropylene tubes (Falcon #2063) by incubating varying amounts of sonicated LPS (obtained from wild type *Salmonella minnesota* and diluted in

RPMI-Hepes [GIBCO/BRL, Gaithersburg, MD]) with 60 ng purified rabbit LBP at 37°C for 30 min. The total volume of the reaction was 100 µl.

Monocytes or neutrophils were added to the pre-formed complexes in the presence or absence of either the monoclonal antibody (MoS39) to human CD14 or of soluble CD14, in a final volume of 400 µl of RPMI-Hepes, and incubated for 3 hours at 37°C in 5% CO_2. Following incubation, viability was 98-100% as determined by trypan blue exclusion.

Activation of the immune system cells was measured by collecting the cell-free supernatants and assaying for TNF activity, using either an ELISA assay (UBI, Lake Success, N.Y.) or a more sensitive MTT cytotoxicity assay. T. Mosmann, J. Immunol. Methods 65: 55 (1983). The latter assay was performed using WEHI-2F cells (provided by Dr. E. Lattime, Thomas Jefferson Medical School, Philadelphia, PA), which are a TNF-sensitive clone of WEHI-164 cells. Briefly, 50 µl of serially diluted cell-free culture supernatants obtained from stimulated neutrophils were added to individual wells in 96 well plates. WEHI-2F cells ($2X10^3$/50 µl of RPMI-HEPES containing 50% FBS) were added to each well. In order to distinguish the cytotoxicity due to TNFa from other cytotoxic activities, the assay was performed simultaneously in the presence of a neutralizing anti-TNFα monoclonal antibody (UBI, Lake Placid, NY) at 1 µg/ml. Following a 36-hour incubation at 40°C [as described by D.R. Branch et al., J. Immunol. Methods, 143:251 (1991)] in 5% CO_2, 25 µl of a solution of 3-[4,5dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide(MTT[5mg/ml, Aldrich, Milwaukee, WI]) prepared in PBS was added and the samples were further incubated for 5 hours at 40°C. Isopropanol containing 0.04 N HCl and 2.5% DMSO (0.15 ml) was added to each well and the resulting formazan crystals were dissolved by vigorous pipetting. The optical density at a wavelength of 570 nm was determined using a Coulter (Hialeah, FL) microplate reader. TNF units were calculated by Probit analysis. R.S. Wallis, J. Immunol. Methods 145:267 (1991). Maximum cytotoxicity was

measured using human recombinant TNFa units detected by this assay was 1 unit/ml.

Figures 1a and 1b show that the LPS:LBP complex stimulates monocytes (Fig. 1a) and neutrophils (Fig. 1b) to release tumor necrosis factor ("TNF") in a dose-dependent fashion.

In figure 1a, PBMC ($1x10^6$) consisting of 1.7×10^5 monocytes (as assessed by morphological examination of WrightGiemsa stained samples) were added to LPS:LBP complexes or to LPS alone and incubated for 3 hours at 37° in 5% CO_2. Cell-free supernatants were assayed for the presence of TNF by the ELISA assay mentioned above.

In figure 1b, neutrophils (3.4×10^6) were added to LPS:LBP complexes or to LPS alone and incubated for 3h at 37°C, 5% CO_2. Since the levels of TNF released by the neutrophils were not detectable by the above ELISA assay, cell-free supernatants were assayed for TNF activity as described above in a cytolytic assay using WEHI-2F cells. No cytotoxicity was detected when WEHI-2F cells were treated with a neutralizing anti-TNF antibody, indicating that all cytotoxicity observed was due to TNF. Data are mean values ± SD of duplicate samples and are representative of four independent experiments.

The monoclonal antibody to CD14 inhibited the secretion of TNF by neutrophils stimulated with LPS:LBP complexes (Fig. 1c). Neutrophils ($3.5x10^6$) were incubated for 30 min at 4°C in the presence of dilutions of $F(ab')_2$ fragments (stock = 0.63 mg/ml) of an anti-CD14 monoclonal antibody or dilutions of F(ab')2 fragments (stock = 0.69 mg/ml) of an isotype-matched irrelevant antibody. LPS:LBP complexes were formed as described above with a final LPS concentration of 5 ng/ml. Antibody-treated cells were added to the LPS:LBP complexes at a final concentration of and incubated for 3 hours at 37°C. The cell-free supernatant was then assayed for TNF activity using WEHI-2F cells. Data shown are mean values of duplicates and are representative of three independent experiments. When error bars are not seen, they

fall within the symbol. The results of this experiment suggest that anti-CD14 antibodies administered *in vivo* might reduce or inhibit the response to LPS.

Figure 1d demonstrates that soluble CD14 will inhibit LPS:LBP-induced monocyte activation (measured by secretion of TNF). For this experiment, monocyte preparations similar to those described in Fig. 1a were treated with recombinant CD14 and were assayed for the production of TNF by the ELISA assay. Results represent duplicates. When error bars are not seen, they fall within the symbol. This result suggests that soluble CD14 administered *in vivo* might reduce or inhibit the response to LPS.

EXAMPLE

sCD14 can protect mice in vivo from LPS-induced death:

The ability of sCD14 to protect animals *in vivo* from the effects of endotoxin-induced septic shock was tested by injecting mice with LPS in the presence and absence of sCD14. Five or six week-old male C57B1/6J mice, weighing approximately 20g each, were injected intraperitoneally with freshly sonicated *Salmonella minnesota* LPS (dosage: 10 μg LPS per gram mouse body weight, supplemented with 68 μg of sCD14, in a final volume of 0.4 mls PBS). Control mice were injected with 10 μg LPS per gram mouse body weight in 0.4 mls PBS in the absence of sCD14. Control and experimental mice were then were observed for a period of seven days.

TABLE 1

LPS	sCD14	%SURVIVAL
10 μ/g body weight (1/5)	0	20%
10 μg/g body weight (3/3)	68 μg	100%

As shown in Table 1, 100% of the mice that were injected simultaneously with LPS and sCD14 survived, as compared to only 20% of the mice that were injected with LPS alone. These results strongly suggest that sCD14 can inhibit the *in vivo* response to LPS including death due to endotoxin shock.

Modifications and variations of the methods for producing preparations of soluble CD14 (for example, the use of different expression systems, such as bacterial expression systems, for expressing recombinant CD14; or the use of different plasmids and viruses than the ones that were used in the examples mentioned below; or the use of nucleic acids that encode proteins that are essentially homologous to the CD14 used herein; or the use of a different isolation and purification scheme that yields a purified product that possesses essentially the same properties of the soluble CD14 described herein) and of the methods for using soluble forms of CD14 to prevent, ameliorate or treat the symptoms of sepsis (such as using a different way of administering the sCD14), will be obvious to those skilled in the art from the foregoing detailed description of the invention. Such modifications and variations are intended to come within the scope of the appended claims.

CLAIMS

We claim:

1. A method for treating symptoms of septic shock in mammals, comprising administering a dose of recombinant CD14 that is effective to treat symptoms of sepsis.

2. A method according to claim 1 wherein said recombinant CD14 is administered by injection.

3. A method according to claim 1 wherein said CD14 is administered by intraperitoneal injection.

4. A method according to claim 1 wherein said CD14 is administered by intravenous injection.

5. A method according to claim 1 wherein said CD14 is administered at the time said mammal is exposed to agents responsible for septic shock.

6. A method according to claim 1 wherein said CD14 is administered at the time said mammal exhibits symptoms of septic shock.

7. A method according to claim 1 wherein said CD14 is administered before said mammal has developed symptoms of septic shock.

8. The method of claim 1 wherein the symptoms of sepsis are caused by gram negative bacteria.

9. The method of claim 1 wherein the symptoms of sepsis are caused by LPS.

10. A method for treating medical conditions that are mediated by the action of CD14, comprising administering a dose of recombinant CD14 that is effective to treat symptoms

of medical conditions that are mediated by the action of CD14.

11. A method according to claim 10 where the medical condition is a member of the group that includes sepsis, gram negative bacteraemia, autoimmune disease and tissue rejection.

12. A method according to claim 10 where the protein is soluble human CD14.

13. A method according to claim 10 where the primary structure of the CD14 is identical to that of the human CD14 molecule, but where the pattern of glycosylation is different.

14. A method according to claim 10 where the CD14 is substantially homologous to that of the human CD14 molecule.

15. An essentially pure preparation of a soluble recombinant protein substantially homologous to human CD14, produced by

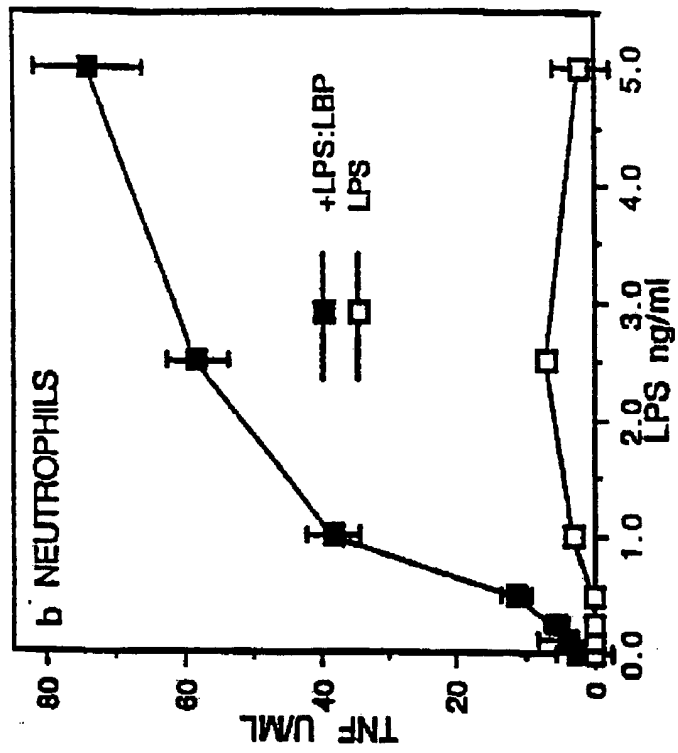
a. expressing an isolated nucleic acid fragment that is substantially homologous to a nucleic acid that encodes human CD14 in an expression system selected from the group of expression systems that comprises plasmids and viruses that can used to express recombinant proteins, and

b. purifying the recombinant protein that is expressed by said expression system.

16. The expression system of claim 15, in which Baculovirus is used to express the recombinant protein.

17. The recombinant protein of claim 15 where the primary structure of the CD14 is identical to that of the human CD14 molecule.

18. The recombinant protein of claim 15 where the primary structure of the CD14 is identical to that of the human CD14 molecule, but where the pattern of glycosylation is different.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+LPS:LBP
LPS
TNF U/ML
0
20
40
60
80
0.0
1.0
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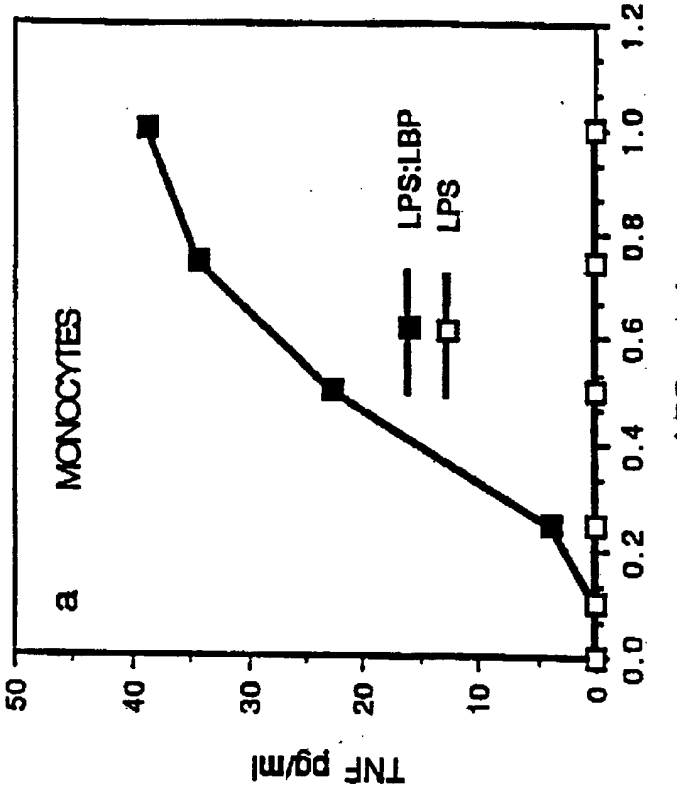
4.0
5.0
LPS ng/ml

Fig. 1(b)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TNF pg/ml
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10
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Fig. 1(a)

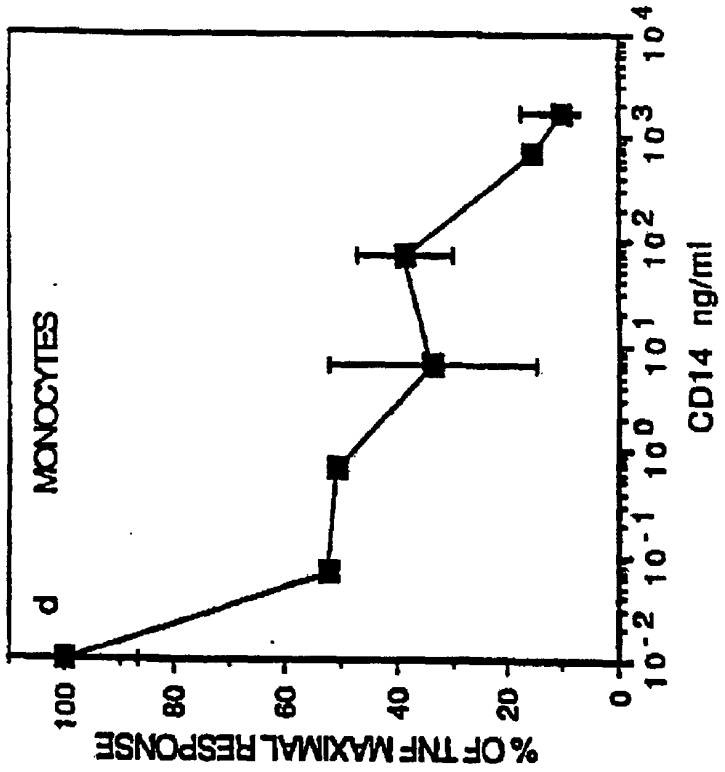
d
MONOCYTES
% OF TNF MAXIMAL RESPONSE
0
20
40
60
80
100
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
CD14 ng/ml

Fig. 1(d)

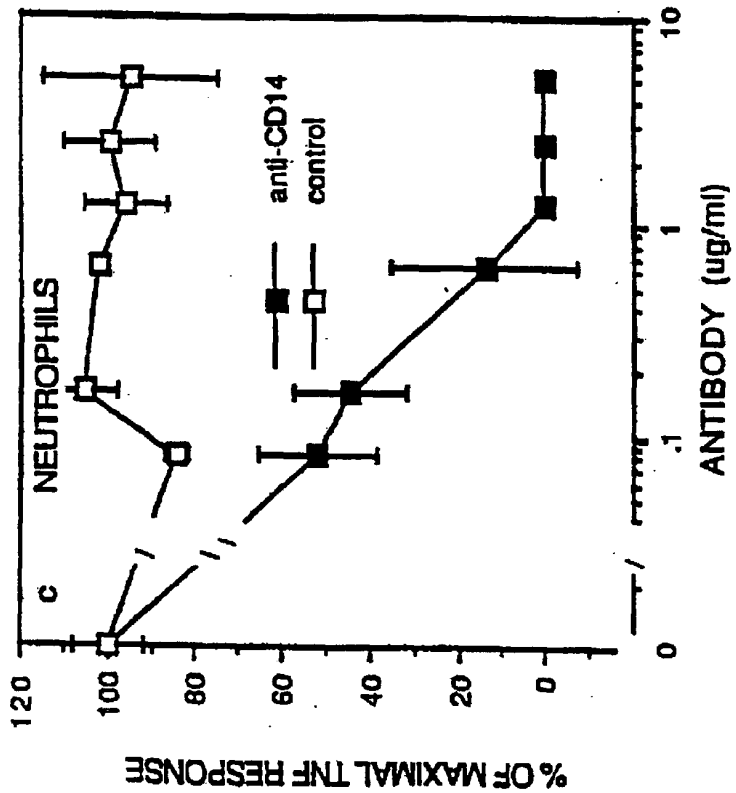
c
NEUTROPHILS
anti-CD14
control
% OF MAXIMAL TNF RESPONSE
0
20
40
60
80
100
120
0
.1
1
10
ANTIBODY (ug/ml)

Fig. 1(c)

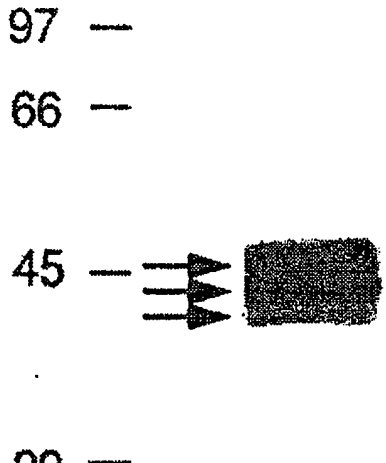

Fig. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/03154

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/02; C07K 13/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12; 530/350, 388.22, 388.23

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG
search terms: CD14, soluble, sepsis, toxic shock

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, A, 91/01639 (ULEVITCH ET AL) 21 FEBRUARY 1991, see entire document, especially page 23, paragraph beginning at line 10.	1-14
X	Nucleic Acids Research, Volume 17(5), issued 1989, K. Matsuura et al, "Nucleotide and amino acid sequences of the mouse CD14 gene", page 2132, entire document.	15-18
Y		1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search 18 May 1993	Date of mailing of the international search report 06 JUL 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer LORRAINE M. SPECTOR, PH.D. Telephone N . (703) 308-0196

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